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New record of *Halla okudai* Imajima, 1967 (Annelida, Eunicida, Oenonidae) from Fukue Island in the Goto Islands, Japan

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Abstract
Halla okudai Imajima, 1967 is an oenonid annelid species that inhabits the intertidal and shallow subtidal zones in China, Malaysia, Australia and Japan. This species is harvested and used as fishing bait in Japan and Malaysia; however, it has been regarded as Near Threatened in Japan based on the categories of the Japanese Red List. Here, we reconstructed the molecular phylogeny of Oenonidae based on 16S rRNA gene sequences. This study provides a new record of *H. okudai* on Fukue Island, in the Goto Islands, Japan.

Keywords
Akamushi, annelid, mitochondrial DNA, muddy sand bottom, Nagasaki Prefecture, polychaete.

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Introduction

Halla okudai Imajima, 1967 (Annelida, Eunicida, Oenonidae; Japanese name: *Akamushi*) is a large polychaete species that can grow up to 900 mm long and 13 mm wide, with over 900 segments, inhabiting the sandy bottoms of intertidal and shallow subtidal zones (Okuda 1933a; Idris and Arshad 2013). The genus *Halla* Costa, 1844 includes two described species, both carnivores and with peculiar feeding behaviors. *Halla okudai* secretes a toxin-containing gel around bivalves and consumes them entirely (Kawai et al. 1999). This interesting feeding behavior has been studied in detail (Imabayashi et al. 1996; Kawai et al. 1999; Saito et al. 2000, 2003, 2004). In addition, its reproduction, early development, growth and regeneration has been previously studied (Itazaki 1982a, 1982b, 1983a, 1983b; Itazaki and Yoshida 1983, 1986a, 1986b).

Halla okudai is geographically distributed in Japan, southern China (Saito et al. 2014), Malaysia (Idris and Arshad 2013) and southern Australia (Paxton 2009). This species is commercially used as fishing bait in Japan and Malaysia (Idris and Arshad 2013; Saito et al. 2014), being quite expensive in Japan [sold at 6,500 Japanese yen (approximately 60$)/100 g] because it is considered the most effective bait worm for fishing Red Seabream, *Pagrus major* (Temminck & Schlegel, 1843) (Saito et al. 2014). *Halla okudai* has been imported from southern China since 2004 (Saito et al. 2011, 2014). On the other hand, the Japanese population of *H. okudai* is considered to be decreasing as its primary prey species, the Manila Clam, *Ruditapes philippinarum* (Adams & Reeve, 1850), is also drastically decreasing (Japanese Association of Benthology 2012; Saito et al. 2014).

This species is listed as Near Threatened according to the Japanese Red List (Ministry of the Environment Government of Japan 2017). *Halla okudai* has been sporadically recorded in Japan, e.g., Enoshima, Kanagawa Pref. (T. Ojima pers. comm.), Bisyamon, Shizuoka Pref. (M. Taru pers. comm.), Lake Hamana, Shizuoka Pref. (Biodiversity Center of Japan 2007), the Seto Inland Sea [stated as the type locality by Imajima (1967), but it is presently considered as the localities of the syntypes in Onomichi, Hiroshima Pref., and Amakusa, Kumamoto Pref. (Tanaka and Sato 2020)] (Okuda 1933a, 1933b; Saito and Imabayashi 1997; Saito et al. 1999, 2007), Hakata Bay, Fukuoka Pref. (Tanaka and Sato 2020), and the Ariake Sea (Itazaki 1982a) (Fig. 1). Information on the geographic distribution of *H. okudai* in Japan is therefore important for conservation of this species. The present study reports a new record of *H. okudai* from sandy bottom on Fukue Island, in the Goto Islands (Nagasaki Pref.), Japan, and a phylogenetic analysis of Oenonidae based on the mitochondrial 16S rRNA sequences to confirm assignment of our specimen to this family.

Methods

Sampling was conducted on September 15th, 2019 at Tomie Bay, Fukue Island, in the Goto Islands. The bottom sediment of this bay is muddy sand with oyster shells and gravels. Some *Zostera* eelgrasses were present. Sediments were collected using a shovel and sieved with a 1 mm mesh. A single large-sized specimen was fixed and preserved in 70% ethanol. The specimen was identified based on the previous descriptions of *Halla okudai* (Okuda 1933a; Imajima 1967; Idris and Arshad 2013). The specimen is deposited in the Seto Marine Biological Laboratory (SMBL), Kyoto University.

We followed Kobayashi (2020) for DNA extraction, PCR and sequencing, except for annealing at 50 °C and the use of the primer pair 16Sann-f/16Sann-r (Kobayashi et al. 2018). The obtained sequence is deposited in the DNA Data Bank of Japan (DDBJ) with DDBJ/EMBL/GenBank accession number LC545402 (345 base pairs). In total, 16S rRNA gene sequences of the two species of Dorvilleidae as outgroup and eight oenonid specimens were included in analysis. Ambiguous positions were eliminated using Gblocks v0.91 (Castresana 2000) with default parameters, except for the option "Allowed gap positions" set to "With half." The GTR + G substitution model was selected based on Akaike information criterion (AIC) using JModelTest v2.1.10 (Darriba et al. 2012). The phylogenetic tree using Bayesian inference was reconstructed using MrBayes v3.1.2. (Ronquist and Huelsenbeck 2003) with 5,000,000 generations (with a sampling frequency of 1,000) and burn-in at 1,250. The maximum likelihood (ML) analysis was performed using IQ-tree (Nguyen et al. 2014) with 1,000 ultrafast bootstrap replicates. The resultant tree was edited using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Order Eunicida
Family Oenonidae
Genus *Halla* Costa, 1844

Halla okudai Imajima, 1967

Halla okudai Imajima 1967: 437; Idris and Arshad 2013: 422–426.
Halla parthenopeia Okuda 1933a: 243–247, 250–251, pl. 12.

Japanese name. Akamushi.

Local name in Malacca, Malaysia. Ruat beting (Idris and Arshad 2013).

New record. Japan • 1 incomplete specimen; Tomie Bay, Fukue Island, Goto Islands, Japan; 32°37′13.0″N, 128°45′49.5″E; approximately 1 m depth; 15 September 2019; Genki Kobayashi, Ryo Mukai, Hajime Itoh leg.; muddy sand bottom; SMBL-V0593; DDBJ/EMBL/GenBank LC545402.

Identification. The specimen lacks an anterior region and the posterior end is about 260 mm in length and 11 mm in maximum width, including parapodia, with

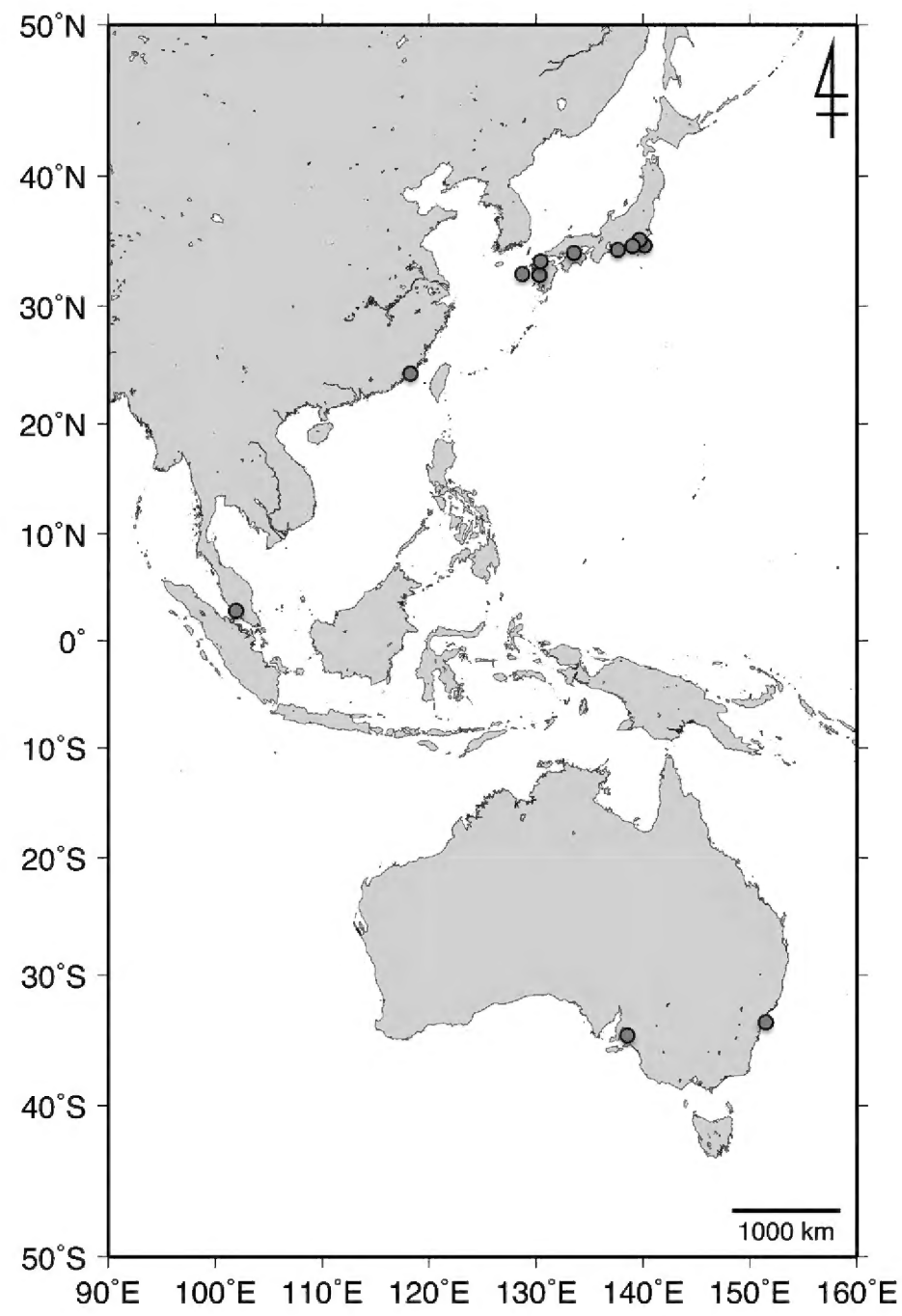

Figure 1. Known distribution of *Halla okudai*. The red circle indicates the record of the present study, whereas yellow circles are records of other reports (Okuda 1933a; Itazaki 1982a; Saito and Imabayashi 1997; Saito et al. 1999, 2007, 2014; Biodiversity Center of Japan 2007; Idris and Arsha 2013; Tanaka and Sato 2020; T. Ojima pers. comm.; M. Taru pers. comm.). The blue circle indicates Xiamen (Amoy), Fuijian Province, China, based on the import information of the species from Xiamen to Japan (Saito et al. 2011).

approximately 530 chaetigerous segments (Fig. 2A). Parapodia are uniramous. Only the capillary chaetae and acicular spines are present (Fig. 2B). The subacicular chaeta includes a simple acicular spine. Dorsal cirri are large, elongated and flattened (Fig. 2B). The morphological characters, especially the large-sized body and flattened oar-shaped dorsal cirri, of the specimen collected on Fukue Island, in the Goto Islands, indicate that this specimen is the posterior part of *H. okudai* (Okuda 1933a; Imajima 1967; Idris and Arshad 2013).

Phylogeny. Monophyly of Oenonidae was highly supported by the molecular phylogeny based on 16S rRNA gene sequences (353 characters after trimming using Gblocks) (Bayesian posterior probability, PP = 1, ML bootstrap value, BS = 99%; Fig. 3). Our specimen was clustered with the *Oenone fulgida* (Lamarck, 1818) clade with weak support (PP = 0.72, BS = 56%) (Fig. 3).

Figure 2. A fragmented specimen of *Halla okudai* collected from Tomie Bay in the Goto Islands, Japan. **A.** Lateral to dorsal view of the specimen. **B.** Parapodia of the specimen. Scale bars: A = 10 mm; B = 1 mm.

Discussion

Genus *Halla* includes two described species; *H. okudai* and *Halla parthenopeia* (Delle Chiaje, 1828). *Halla okudai* was described based on the following morphological differences from *H. parthenopeia*: (1) large-sized body; (2) subtriangular prostomium; (3) digiform nuchal antenna; and (4) the subacicular chaeta first appears at the 70th segment (Imajima 1967). However, recently, *H. parthenopeia* as large as *H. okudai* has been collected (Osman et al. 2010) and the possibility of synonymy of *H. okudai* has been proposed based on morphological observation of both species (Idris and Arshad 2013). Nonetheless, the type locality of *H. parthenopeia* (Naples, Italy) is far from that of *H. okudai* (Japan). Among oenonids known from Japan, *Oenone fulgida* resembles *H. okudai* but these two species are distinguishable because bidentate hooded hooks are present in *O. fulgida* but not in *H. okudai* (Imajima 1967; Zanol and Ruta 2015). Based on these reasons, we identified the present specimen as *H. okudai*.

Molecular phylogenetic analysis, the first including the species of *Halla*, also supported the idea that our specimen belongs to Oenonidae (Fig. 3). Unfortunately, the phylogenetic position of *Halla* was unclear. However, the close relationship among *Halla*, *Oenone* Lamarck, 1818 and *Tainokia* Knox & Green, 1972 implied by the present result is congruent with the morphological similarity (Orensanz 1990, Paxton 2009); these genera were previously recognized as "Lysaretidae," which has been synonymized with Oenonidae.

Although *H. okudai* is widely distributed in the western Pacific, for example, Japan, China, Malaysia and Australia (Paxton 2009; Idris and Arshad 2013; Saito et al. 2014), it is sporadically occurring in Japan (see Introduction; Fig. 1). The present study provides the westernmost record of *H. okudai* in Japan. Also, this is the first record of this species in Nagasaki Prefecture. Several previous studies have stated or experimentally shown that *H. okudai* feeds on bivalves (Okuda 1933b; Itazaki 1982b; Saito and Imabayashi 1994; Imabayashi et al. 1996). We observed at least five species of the following bivalve species during our survey in Tomie Bay: *Fulvia hungerfordi* (G.B. Sowerby III, 1901) (Cardiidae), *Macoma incongrua* (Martens, 1865), *Nitidotellina lischkei* M. Huber, Langleit & Kreipl, 2015 (Tellinidae), the Japanese pearl oyster *Pinctada imbricata* Röding, 1798 (Margaritidae), and the razor clam *Solen strictus* Gould, 1861 (Solenidae). Although *H. okudai* is shown to possess strong preference on *R. philippinarum* (Saito and Imabayashi 1994), this bivalve species was not observed during our survey. Absence of *R. phillippinarum* in the area where *H. okudai* was collected has been reported (Itazaki 1982a).

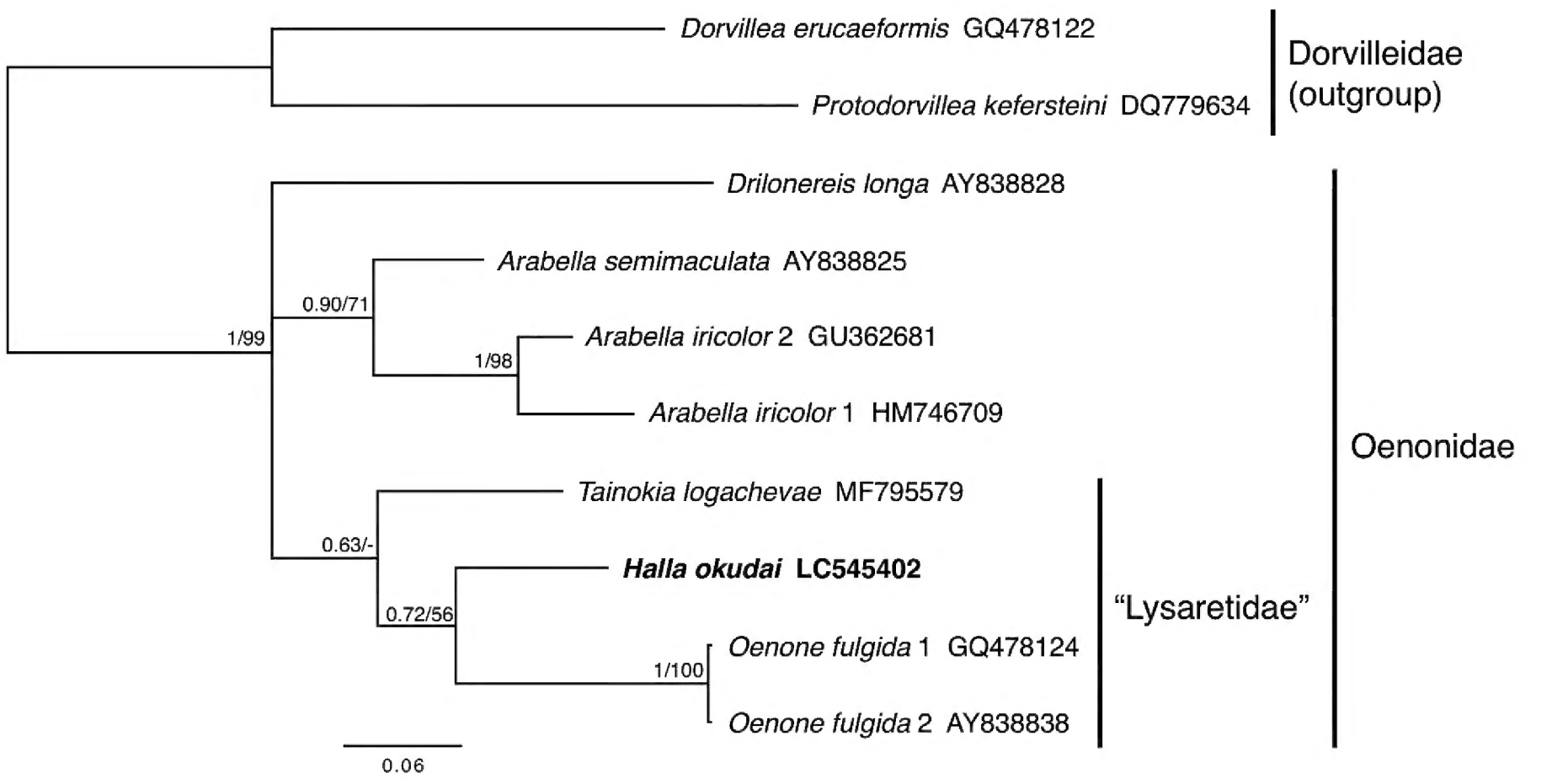

Figure 3. Bayesian phylogeny of Oenonidae based on 16S rRNA gene sequences (353 characters). Species names are followed by GenBank accession numbers. The numbers above the branches indicate posterior probability, followed by percentage of maximum likelihood bootstrap values above 50% (a hyphen represents <50%). *Halla okudai*, whose nucleotide sequence was newly obtained, is shown in bold. "Lysaretidae" has been synonymized with Oenonidae.

The present record of *H. okudai* is valuable because this species is sporadically recorded and ranked as Near Threatened in Japan (Ministry of the Environment, Government of Japan 2017). Japan may represent the northern edge of the geographic distribution of *H. okudai*, as populations at the range margin of species are typically fragmented and smaller (Bridle and Vines 2007). Geographically wide-ranging *H. okudai* possibly includes distinct species that do not show clear morphological differences, and this species may actually be in danger of extinction. More information about the geographical distribution of *H. okudai* in Japan would be needed to evaluate the vulnerability of Japanese population.

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Authors' Contributions

GK conceived the research question. GK, RM, HI did field survey. GK collected data and wrote the first draft of the manuscript. RM identified bivalve species. All authors reviewed and agreed on the final version of the manuscript.

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